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Project Director: Dr. R. M. Nerem School/Lab Mechanical Engineering

Sponsor: The University of Texas Health Science Center at San Antonio

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Title: Cellular Mechanisms in Atherogenesis

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ATTN: Mr. E.A. Seibold
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See Attached ____ Supplemental Information Sheet for Additional Requirements.

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1. GT and UTHSCSA shall determine disposition of patents based on the percentage of contribution of each party.
2. Rebudgeting between budget catergories requires sponsor approval.

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12 / 88

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NOTICE OF PROJECT CLOSEOUT

Date 12/14/88

Project No. E-25-606 Center No. R6286-0A0

Project Director R.M. Nerem School/Lab ME

Sponsor The University of Texas Health Science Center at San Antonio

Contract/Grant No. Agreement dtd. 2/26/87 GTRC XX GIT ____

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Effective Completion Date 6/30/88 (Performance) 8/30/88 (Reports)

Closeout Actions Required:

____ None
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Nerem, Robert M. | 334-30-6030

C. PROGRESS

Introduction

The work reported in this section was supported as part of a Program Project entitled "Cellular Mechanisms in Atherogenesis" (HL-26890). The goal of the project was to examine the structural and functional consequences of the application of fluid-mechanically imposed shear stress on vascular endothelial cells. The general question being addressed by this work was "does a fluid-mechanically imposed shear stress alter the structure and function of endothelial cells?"

Efforts on this previous grant have been directed mostly to the use of cultured bovine aortic endothelial cells. The focus of these studies has been on the investigation of the response of confluent, cultured endothelial cells to known levels of shear stress using a parallel plate, channel flow device which is described under **METHODS.** The emphasis has been primarily on the influence of shear stress on the orientation and elongation of endothelial cells, on the mechanics of the deformation to stress, and on changes in electrophysiological properties as reflected in membrane potential. The range of conditions to which we have exposed bovine aortic endothelial cells includes shear stresses from 0 - 90 dynes/cm^2, exposure durations up to 72 hours, and two different substrates, glass and Thermanox.

The use of the parallel plate, channel flow shear stress device (Figure 1) offers several advantages. First, it provides an extremely simple, well-defined fluid mechanical environment where we know the shear stress to which the cultured endothelial cells are being exposed. Not only is this flow geometry amenable to mathematical analysis, but flow visualization studies have demonstrated that the streamlines are straight and parallel and thus that the flow is what it is supposed to be.

Secondly, with the parallel plate, channel flow device and with the Thermanox plastic substrate, we have conducted studies at a shear stress as high as 90 dynes/cm^2. To our knowledge, this is the highest shear stress by far at which cell culture data has been obtained. Furthermore, it is entirely possible that we will be able to go to even higher shear stress levels. Since in vivo peak shear stress values may range up to 100 dynes/cm^2, the conditions of these laboratory studies are physiologic from the viewpoint of the stress levels to which the cells are exposed.

Finally, the simple parallel plate, channel flow device used here is sufficiently miniaturized that it can be placed on the stage of a light microscope for continuous, phase contrast observation. Thus, as the cells orient and elongate with time, they are under continuous observation. In our laboratory, there is a TV monitor system connected to the light microscope, which with a time-lapse video recorder allows even better monitoring of the slow changes in cell geometry and orientation which take place in response to mechanical stress.

Endothelial Cell Geometry and Orientation

In initiating our studies of the effect of shear stress on confluent monolayers of cultured BAEC using the parallel plate flow chamber, we first focused on changes in cell shape and orientation[34]. Figure 2 shows a population of bovine aortic endothelial cells, exposed in this device to a constant shear stress, as compared with a similar, but control population of cells. As may be seen, there is an orientation of the cells exposed to shear which is not present for controls; however, this effect is highly dependent on such parameters as the level of shear stress (Figure 3A), the duration of exposure, and the substrate. Furthermore, the time history of the orientation process indicates that cultured endothelial cells exposed to shear first begin to elongate and then, and only then, start to orient themselves with the flow.

Cell elongation and orientation also has been studied for EC treated with cytochalasin B, a cytoskeletal inhibitor which disrupts actin assembly. Initial results for shape index (4π area/perimeter^2) are presented in Figure 3B. Here cytochalasin B was introduced into the perfusing media at a concentration of 2 μ molar, either during the first 24 hours of exposure to shear stress (τ_w + CB) or after 24 nours of pre-stress, followed by another 24 hours of concurrent exposure to shear stress and cytochalasin B (τ_w + (τ_w + CB)). In either case, the influence of concurrent exposure to shear stress and cytochalasin B was to produce higher values of the shape index; i.e., a more rounded endothelial cell.

Cytoskeletal Element Localization

As an initial study of cytoskeletal structure, actin filaments were stained with rhodamine phalloidin (Molecular Probes, Inc., OR), which is specific for F-actin, and viewed using fluorescent microscopy. For short exposure times to shear stress, a change in cellular morphology was not observed with phase contrast optics. However, it was observed that the microfilament system was affected, with the dense peripheral band mostly disappearing and an increase in stress fibers shown in all cases. These stress fibers have a more organized appearance, and it appears that the increase in stress fibers may be shear-stress dependent.

At 4 hours of exposure to shear stress, there is an increased number of stress fibers aligned with the flow field. With a longer exposure time of 12 hours the stress fibers are mostly aligned with the direction of flow. At 24 hours of exposure to shear stress, in all three cases, i.e., 10, 30, and 85 dynes/cm^2, stress fibers are aligned with the direction of flow. For the highest shear stress level, a higher concentration of microfilament bundles also existed at the cell periphery. Figure 4 shows photomicrographs of rhodamine phalloidin stained EC on Thermanox for no-flow, control conditions and for exposure to 85 dynes/cm^2 for 24 nours.

Cell Mechanical Properties

The data that we have obtained on endothelial cell shape and orientation suggest that the response of an endothelial cell to a shear stress environment, may depend on the cell's mechanical and motile properties. Thus experiments were initiated in our laboratory to study endothelial cell deformation using a

micropipette technique. In brief, a small micropipette (inside diameter - 3μm) is attached to a pressure reservoir. The tip of the pipette is brought close to the cell. When the pipette makes contact with the cell, the pressure reservoir is lowered to produce a negative pressure on the cell surface, and the cell is pulled into the pipette. The elongation of the cell inside the pipette in relation to pressure is recorded. Then the pressure is increased, and the cell de-elongation in relation to pressure is measured until the cell is removed from the pipette.

For a cell which has never been exposed to flow, the cell becomes spherical upon detachment. F-actin staining allows one to visualize a submembranous cortical cytoskeletal layer, and we believe that it is this layer which represents the primary load-bearing component in a micropipette stress-strain measurement[35]. By using a membrane-like model for this cortical layer, one can determine an effective cortical layer shear modulus (μ) which represents the strength of this cortical cytoskeletal layer. Figure 5 presents results for the cortical layer shear modulus for cells detached from two different substrates where in both cases the monolayers were confluent and under no-flow conditions. As may be seen, cells detached from a Thermanox substrate have a shear modulus which is twice that of cells detached from glass. This suggests that different substrates induce cells to develop different cytoskeletal structures and thus exhibit different mechanical properties. This is consistent with the post-injury motility studies of Young and Herman[26] which demonstrate that for different extracellular matrix materials, there are differences in EC stress fiber density and migration velocities following monolayer injury.

The importance of cytoskeletal elements in the determination of the cortical layer shear modulus also is shown in Figure 5 where values of μ are shown for control cells on a Thermanox substrate as well as for cells treated with cytochalasin B and colchicine. Cytochalasin B disrupts actin assembly, and as may be seen in Figure 5, the cortical layer shear modulus is an order of magnitude less than that for control cells. Colchicine affects microtubular assembly, and again there is a dramatic reduction in shear modulus, although not as great as that due to cytochalasin B.

We have also studied endothelial cells exposed to shear stress[36]. As seen in Figure 6, an endothelial cell, which is exposed to a high shear stress for a long period of time and which is subsequently mechanically detached, retains its elongated shape. This is illustrated in Figure 6B and may be contrasted with Figure 6A where a spherically-shaped cell detached from a control, no-flow monolayer is shown. Because of the elongated shape of EC detached after exposure to shear stress, together with their altered cytoskeleton and the concept of a cortical layer, a membane-like model of shear modulus is no longer strictly applicable. Thus, the micropipette data from EC exposed to shear and then detached has been analyzed using a mechanical stiffness parameter, K. This parameter is defined as the ratio of $\Delta p \times R$, the tension being applied to the cell surface, divided by L/R where L is the elongation of the cell in the micropipette and R is pipette radius. As may be seen in Figure 7, with increasing level of shear stress and/or increasing exposure time, there is an increase in cell stiffness as measured by the parameter, K. This suggest that, in response to shear stress, i.e., as an adaptation to a stress environment, the endothelial cell reorganizes its internal cytoskeletal structure. Thus, both our observations of F-actin and our measurments of EC mechanical properties support the notion that

endothelial cells, in response to shear stress, adapt their cytoskeletal structure to this flow environment. Furthermore, this adaptation is initiated prior to any observable changes in cell shape and orientation.

As an alternate approach to the analysis of micropipette data obtained from EC exposed to shear stress, a model which treats the sheared elongated cells as a homogenous body with an effective Young's modulus may be more appropriate. The details of this analysis are contained in the **METHODS** section. Applying the resulting equations to the sheared EC micropipette data, characteristic values of Young's modulus, E, on the order of 10,000 dynes/cm^2 are calculated for EC exposed to 30 or 85 dynes/cm^2 for 24 hours. This is as compared to values of E of 3,000 dynes/cm^2 (or less) for cells from control, no-flow conditions.

Intracellular Potential

The relation between the intracellular potential of vascular endothelial cells and wall shear stress was studied in vitro by exposing cultured bovine aortic endothelial cells to known levels of a fluid-imposed shear stress using the parallel plate flow device. Endothelial cells were exposed to shear stress for either 5 or 25 hours. The intracellular potential recordings were obtained from two regions of cells (cytoplasm and nucleus) using glass microelectrodes. Results obtained from several hundreds of cells under control conditions indicate that the mean value for the intracellular potential of cytoplasmic regions is 2.7 mv (inside negative) and for nuclear regions it is 13 mv (inside negative). The difference between these two means is significant (P-value = 0.001, U-test) It was found that after exposure to shear stress, the magnitude of the intracellular potential increased significantly with duration of exposure and level of shear stress. Even after a short exposure time of five hours at a shear stress level of 15 dynes/cm^2, a small but significant increase in intracelluar potential was observed. However, intracellular potentials from nuclear regions did not increase as much as those from cytoplasmic regions. The most significant increase in cytoplasmic intracellular potential was observed at a shear stress level of 45 dynes/cm^2. After 25 hours of exposure to 90 dynes/cm^2, intracellular potential mean values for the cytoplasm and nuclei were 33 and 21 mv respectively (inside negative). Results for the cytoplasm are presented in Figure 8A.

A key question is, if the shear stress is removed and the EC monolayer placed under a no-flow, post-shear condition, are shear-induced changes reversible? If so, what is the time course of events? Figure 8B presents post-snear intracellular potential values for a confluent monolayer which has been exposed to 60 dynes/cm^2 for 25 hours. As may be seen, there is a gradual decline in intracellular potential with time. It appears that approximately 50 hours is required to reach a control value of 2-3 mv (inside negative). This is significantly longer than the time required for the cell's mecnanical stiffness to relax, the latter being on the order of 20 hours. This suggests mechanisms, which though quite likely related, are not one and the same.

Finally, measurements of intracellular potential at short exposure times, e.g., a 1/2 hour exposure to shear stress, indicate that changes in intracellular potential occur quite early, i.e., on the same time scale as that associated with the early changes in cytoskeletal structure. Since these measurements may

reflect membrane ion transport and/or intracellular Ca^{++} mobilization, it may well be that these ion mechanisms are inherent messengers in the cell's recognition and transduction of a shear stress signal.

LDL Endocytosis Under the Infuence of Shear Stress

Studies on the endocytosis of LDL under the influence of shear stress have been initiating using ^{125}I-LDL and two parallel plate, channel flow devices in series. The one channel flow device has a channel height of 250 microns, and with the flow rate used, this results in the confluent monolayer of endothelial cells being exposed to a shear stress of 30 dynes/cm^2. The other channel flow device has a channel height which is large such that, with the flow rate used, the endothelial cells are exposed to a shear stress of less than 1 dyne/cm^2. The latter serves as a control, and this experimental design has been employed so that each high shear stress test condition has its own control. This allows a paired t-test to be used for statistical analysis of the data.

An illustration of the experimental set-up is presented in Figure 1B, and as may be seen, both temperature and CO_2 content of the perfusing culture media is monitored and maintained. Shown in Figure 1B is both the high shear (S) and low shear, control (C) channel flow devices. The former is continuously monitored with a TV camera/monitor system. The design of the channel flow device is such that each channel flow device contains four ports. This allows multiple measurements to be carried out during each experiment, e.g., data for different exposure times can be obtained from a single experiment by removing coverslips at selected time points. The cell protein content has been measured as an indicator of the number of cells per coverslip sample, which has been used to normalize the ^{125}I-LDL data. Also, measurements of the ^{125}I-LDL concentration in the perfusing culture media have been carried out throughout each experiment, and this has been used to normalize for variations in media concentration from one experiment to the next.

Based on the time required for a confluent layer of endothelial cells to elongate and orient, two different types of experimental conditions have been investigated, although both at an experimental shear stress(S) of 30 dynes/cm^2. These are are termed pre-stress and concurrent stress respectively. The pre-stress condition is one where the endothelial cells are exposed to a shear stress for 24 hours prior to the introduction of the ^{125}I-LDL. This means that the cells have already oriented themselves with the flow and undergone elongation before exposure to ^{125}I-LDL. The concurrent stress condition, on the other hand, is one where the ^{125}I-LDL is introduced at time zero, i.e., at the initiation of the exposure of the endothelial cells to shear stress. The result is that, during LDL-endocytosis, the endothelial cells are undergoing elongation and orientation, i.e., in the process of undergoing dynamic changes in shape and orientation.

For both prestress and concurrent stress experimental regimens, ^{125}I-LDL internalization studies were performed in the presence of circulating medium containing 20% whole fetal calf serum. This was done to determine whether shear stress levels could influence LDL endocytosis without manipulating LDL receptor expression through the use of lipoprotein deficient serum. In the first series of experiments the BAEC cultures were incubated with ^{125}I-LDL under continuing

shear stress after shear stress-induced changes in cell shape and orientation were largely complete. BAEC cultures were exposed to a wall shear stress of 30 (HS) and <1 dyne/cm^2 (LS) for 24 hr, after which the ^{125}I-LDL was added to the perfusate, and the shear stress continued for 2 and 24 hr respectively, at which time ^{125}I-LDL internalization was measured. Eight HS and LS experiments were performed in duplicate, and the results are presented in Figure 9. To permit comparison and analyses of the results from the individual experiments, the data was normalized by expressing the ^{125}I-LDL internalization observed as a ratio of HS to LS within each experiment. Internalization of ^{125}I-LDL was significantly greater in BAEC exposed to a high wall shear ($p=0.018$) compared to a low shear when the ^{125}I-LDL was present in the perfusate for 24 hr. When ^{125}I-LDL was present for only 2 hr, there was a trend for the HS:LS ratio to be greater than 1, but the difference did not attain statistical significance ($p>.10$).

The second series of experiments was undertaken to determine the influence of wall shear stress on ^{125}I-LDL internalization during shear-induced changes in cell shape and orientation, i.e., concurrent stress. BAEC cultures were exposed to both HS and LS for 2 and 24 hr, with ^{125}I-LDL present in the perfusate throughout the application of shear. Six duplicate experiments were performed. Although the ratio HS:LS was greater than 1 at 2 hr (1.25),this difference did not attain statistical significance ($p>.10$) (Figure 9). At 24 hrs, however, concurrent stress resulted in a statistically significant enhancement of ^{125}I-LDL internalization ($p=0.028$).

Both the above studies establish that the application of laminar steady state wall shear stress to confluent BAEC cultures enhances the internalization of ^{125}I-LDL, and further that this effect is not dependent upon prior shear-induced changes in cell shape and orientation, nor is it limited to the time period during which cell geometry is changing. These studies also indicate that this enhanced ^{125}I-LDL uptake occurs even in the presence of lipoprotein containing fetal calf serum, i.e., down regulated cells.

Based on our findings that ^{125}I-LDL internalization was increased in BAEC exposed to high wall shear stress, a series of experiments was performed to determine whether this enhanced LDL internalization might be mediated via an influence of shear stress on the LDL receptor. Accordingly, confluent BAEC on coverslips were exposed to HS, LS, or NS for 24 hours in the presence of circulating medium containing 20% LDS to maximize LDL receptor expression. At the end of the shear stress period, the BAEC were removed and LDL receptor status was assessed by incubating the cells with ^{125}I-LDL at 37°C for 2 hours to measure both receptor- and non receptor-mediated ^{125}I-LDL internalization and degradation. Specific or receptor-mediated internalization and degradation were determined as the difference measured in the presence and absence of an excess of unlabeled LDL.

Receptor-mediated internalization of ^{125}I-LDL measured immediately after removal of coverslips from shear stress conditions was significantly enhanced in BAEC exposed to HS relative to BAEC exposed to NS (Figure 10). Though the overall means of ^{125}I-LDL internalization in BAEC exposed to LS or NS in 9 experiments were identical (56 ng ^{125}I-LDL/mg protein), analysis of the results using Student's paired t-test failed to demonstrate a significant difference ($p=0.07$) between BAEC exposed to HS compared to LS. On the other hand, receptor-mediated degradation of ^{125}I-LDL was significantly greater ($p<.01$) in HS treated BAEC

compared to either LS or NS treated cells (Figure 10). No significant difference in ^{125}I-LDL internalization or degradation was found between LS or NS treated BAEC when subjected to paired analysis ($p>.20$). Similarly, non-specific or non-receptor-mediated ^{125}I-LDL uptake and degradation by BAEC was not influenced by shear stress levels (Figure 10).

To determine if shear stress influenced the availability of cell surface ^{125}I-LDL specific binding sites or receptors, ^{125}I-LDL binding was measured at 4°C over 2 hours immediately after removal from shear stress in 6 replicate experiments. As illustrated in Figure 10, receptor-mediated or specific ^{125}I-LDL binding was significantly increased in BAEC exposed to HS relative to either LS or NS ($p<.01$). Further, though the overall mean of receptor-mediated ^{125}I-LDL binding to LS-treated BAEC was enhanced compared to BAEC exposed to NS conditions, this apparent difference was not verified statistically using Student's paired-t-test ($p>.10$). Similar to studies at 37°C, nonspecific binding of ^{125}I-LDL at 4°C to BAEC, measured in the presence of excess unlabeled LDL, was not influenced by exposure to shear stress.

It is of considerable interest that a shear stress of 30 dynes/cm^2 enhances receptor-mediated binding and degradation of LDL in confluent BAEC cultures. This influence of shear on endothelial LDL receptor expression and function could result from an increase in receptor number, enhanced exposure of existing receptor domains thus facilitating ligand-receptor coupling, enhanced receptor affinity, or an accelerated recycling of receptors to the plasmalemmal membrane. These various possibilities are presently being explored. It is clearly of importance to understand how a fluid mechanical shear stress signal is translated into the heightened LDL receptor expression and function we have observed. Of particular importance is our observation that a factor other than the availability of exogenous cholesterol may also regulate cellular LDL receptor expression and function.

Cell Proliferation

In order to determine whether or not the observed enhancement in ^{125}I-LDL endocytosis was possibly due to shear-induced EC replication, confluent EC cultures were exposed with 2 μCi of ^{3}H-thymidine. The results are summarized in Table 1, where it can be seen that ^{3}H-thymidine incorporation into endothelial cell DNA was not significantly different among the HS, LS or NS cultures. In particular, exposure to a steady state high wall shear of 30 dynes/cm^2 for 24 hr did not result in a measurable increase in EC replication, making it unlikely that the enhanced ^{125}I-LDL endocytosis is the result of shear-induced BAEC replication. Cell replication rate as measured by ^{3}H-thymidine autoradiography confirmed these results.

Studies on the influence of shear stress on cell proliferation in sub-confluent EC monolayers were also undertaken. These were carried out by measuring cell density as a function of time. Typical growth curves for EC on glass and Thermanox are shown for no-flow, control conditions in Figure 11A. In the exponential period of growth, the doubling time is approximately 20 hours. Contact inhibition produces a plateau in the growth curve, with the cell density being slightly higher on glass than on Thermanox.

TABLE 1

^{3}H-Tnymidine incorporation into BAEC DNA after 24 hr exposure of confluent cultures to HS (30 dynes/cm^2), LS (< 1 dyne/cm^2) or no shear (NS)

	^{3}H Tnymidine Incorporation (dpm/10^3 cells)		
	HS	LS	NS
No. of Experiments (n)	3	4	4
Mean ± SEM	13.6 ± 2.7	13.5 ± 1.40	12.0 ± 1.1

In the presence of shear stress, the proliferation of EC on Thermanox is altered as shown in Figure 11B. For low shear stresses, i.e., 5 dynes/cm^2 or less, any detectable effect is absent as was noted in the earlier study of Dewey, et al.[25]. However, for shear stresses in the range of 30 to 90 dynes/cm^2, there is a drammatic effect. Cell proliferation is significantly slowed, with the growth at 90 dynes/cm^2 being almost totally arrested. These cell density studies have been confirmed with ^{3}H-thymidine in corporation measurements. However, the mechanism(s) involved in this remain to be explored.

Pulsatile Shear Stress Effects

During the past 12 months, our attention has focused increasinlgy on the effects of pulsatile shear stress on cultured bovine aortic endothelial cells. In vivo the flow waveform and therefore the shear stress waveform is pulsatile. It is thus important to determine the sensitivity of EC responses to pulsatile shear stress. Of particular interest are the following three questions: i) what is the frequency response characteristics of EC exposed to pulsatile shear stress, ii) are different biological aspects of the total EC response sensitive to different frequencies or frequency ranges, and iii) are there any differences associated with a reversing shear stress waveform as opposed to one which is non-reversing.

Our initial pulsatile flow experiments have focused on cell shape and orientation, cell mechanical properties, and intracellular potential. The flow environment has included a mean shear stress upon which is super imposed a 1 Hz pulsatile component. In the presence of a pulsatile flow, where there is both a mean shear stress, τ_w, and a pulsatile component, thus producing maximum and minimum values, $\tau_{w_{max}}$ and $\tau_{w_{min}}$ respectively, EC are found to be more elongated. This is true when compared with steady flow results at a shear stress equivalent to the mean value, τ_w. It is also true when compared with steady flow results at a shear stress equal to the maximum shear stress value $\tau_{w_{max}}$. The latter suggests that the elongation observed for pulsatile flow is not just due to an elevated level of shear stress over some portion of the pulsatile flow cycle, but rather because of a change in the basic interaction in the presence of pulsatility.

Initial results indicate that in the presence of pulsatile flow EC exhibit an enhanced cell stiffness, as illustrated in Figure 12A, and an increase in intracellular potential as shown in Figure 12B. In Figure 12B, the intracellular potential in the presence of pulsatile flow is enhanced over that found for steady flow at a shear stress equal to the mean value, τ_w, but less than that for steady flow at a shear stress equal to the maximum value, $\tau_{w_{max}}$. This is in contrast to the pulsatile flow effect on cell elongation and suggests that we have much to learn about the response and adaptation of EC to a pulsatile flow environment.

Summary of Progress

The overall aim of this project has been to examine the structural and functional consequences of the application of a fluid-imposed shear stress to cultured endothelial cells. The studies employ a parallel-plate flow chamber with bovine aortic endothelial cells being exposed to a laminar shear stress. During the five years of this project, the research has evolved from initially looking at enface cell morphology, e.g., cell shape and orientation, to recent studies focusing on changes in cell structure and function. Our understanding of the response of EC to a fluid-imposed shear stress may be summarized as follows:

the elongation and orientation of a confluent EC monolayer exposed to a steady shear stress is dependent on the level of the stress, the duration of exposure, and the nature of the substrate;

although such enface morphological changes are the most visible feature of the response of EC to shear stress, there are alterations in F-actin distribution and intracellular potential which precede any detectable change in shape, which itself precedes any change in cell orientation;

changes in EC cytoskeletal structure, e.g., F-actin, appear to be reflected in EC mechanical properties as determined using a micropipette technique and whereby cells in response to shear stress are observed to become stiffer;

whereas in response to exposure to a steady shear stress, the nonspecific component of ^{125}I-LDL binding, internalization, and degradation by EC is unchanged, the specific, receptor-mediated component is significantly enhanced;

this enhancement of a receptor-mediated process cannot be ascribed to increased cell proliferation since thymidine incorporation measurements show no change relative to a control, no-flow confluent monolayer; however, for a non-confluent monolayer, there is an effect of shear stress on cell proliferation with the cell doubling time increasing with increasing shear stress; and

the superposition of flow pulsatility upon a steady shear stress results in increased EC elongation, an enhancement in cell mechanical stiffness, and a further hyperpolarization of the cell membrane.

A basic question emanating from these observations is how does an endothelial cell recognize a shear stress signal, discriminate between difference in shear stress waveform shape, and then transduce the signal into a change in cell function? It seems reasonable to hypothesize that an endothelial cell's recognition of shear stress is a direct membrane effect. This could be due to mechanosensitive membrane ion channels or due to some other effect on the membrane. A possible example of the latter would be an influence of shear stress on membrane phosphoinositol turnover. In this case the transduction of the signal would involve the transient production of diacylglycerol which in turn activates protein kinase C. Diacylglycerol normally is virtually absent from membranes, but in response to an extracellular signal is transiently produced and then is further degraded to arachidonic acid for thromboxane and prostaglandin synthesis.

The intracellular mobilization of Ca^{++} also could serve as a second messenger. This could be in place of the phospnoinositol signal transduction pathway or could occur concommitantly, with the two second messengers acting synergistically. It is well known that eukaryotic cells, when exposed to a change in chemical environment, undergo a rapid elevation in intracellular Ca^{++} concentration. The most common translation of the Ca^{++} signal is calmodulin, with many of the Ca^{++} effects being exerted through calmodulin-regulated enzymes. What is not known is whether such a response can be elicited by a change in a cell's mechanical environment.

The experiments proposed as part of this effort are designed to provide further insight into the response of EC to the mechanical environment provided by a fluid-imposed shear stress and the mechanisms involved in the signal recognition/transduction process.

List of Publications from Research on Grant HL-26890

Journal Articles--

Nerem, R.M., "Atherogenesis: Hemodynamics, Vascular Geometry, and the Endothelium," Biorheology, Vol. 21, 1984, pp. 565-569.

Levesque, M.J. and Nerem, R.M. "The Elongation and Orientation of Cultured Endothelial Cells in Response to Shear Stress," ASME J. Biomechanical Engineering, Vol. 107, No. 4, 1985, pp. 341-347.

Levesque, M.J., Lipesch, D., Moravec, S. and Nerem, R.M., "Correlation of Endothelial Cell Shape and Wall Shear Stress in a Stenosed Dog Aorta," Arteriosclerosis, Vol., 6, No. 2, 1986, pp. 220-229.

Sato, M., Levesque, M.J. and Nerem, R.M., "Application of the Micropipette Technique to the Measurement of the Mechanical Properties of Cultured Bovine Aortic Endothelial Cells,", ASME J. Biomechanical Engineering, Vol. 109, No. 1, 1987, pp. 27-34.

Sato, M., Levesque, M.J. and Nerem, R.M., "Micropipette Aspiration of Cultured Bovine Aortic Endothelial Cells Exposed to Shear Stress," Arteriosclerosis (in press).

Sprague, E.A., Steinbach, B.L., Nerem, R.M. and Schwartz, C.J., "Influence of a Laminar Steady State Fluid-Imposed Shear Stress on the Binding, Internalization, and Degradation of Low Density Lipoprotein (LDL) by Cultured Arterial Endothelium", Circulation (tentatively accepted, subject to revisions).

Levesque, M.J., Sato, M. and Nerem, R.M., "Micropipette Aspiration of Cultured Bovine Aortic Endothelial Cells: Effect of Cytoskeletal Disrupting Agents," (submitted for publication).

Levesque, M.J., Sprague, E.A. and Nerem, R.M., "Proliferation of a Non-Confluent Monolayer of Cultured Bovine Aortic Endothelial Cells Exposed to a Steady-State Laminar Shear Stress," (submitted for publication).